THE EFFECT OF DIPINE ON PHENOBARBITAL INDUCTION OF CYTOCHROME P_{450} INDUCTION AND STIMULATION OF MITOTIC ACTIVITY IN THE HEPATIC CELLS OF THE RAT

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Translation of: "Vliyaniye Dipina na Induktsiyu Fenobarbitalom Tsitokhroma P_{450} i Stimulyatsiyu Mitoticheskoy Aktivnosti v Kletkakh Pecheni Krysy," Farmakologiya i Toksikologiya, Vol. 36, No. 5, 1973, pp. 597-599.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 DECEMBER 1973

STANDARD TITLE PAGE

1. Report No. NASA TT F-15,225	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle THE EFFECT OF DIPINE ON PHENOBARBITAL INDUCTION OF CYTOCHROME P_{450} INDUCTION AND STIMULATION OF MITOTIC ACTIVITY IN THE HEPATIC CELLS OF THE RAT		5. Report Date DECEMBER 1973 6. Performing Organization Code
7. Author(s) V. V. Klimenko, L. Ye. Nemirovskiy, G. R. Mutovin		8. Performing Organization Report No. 10. Work Unit No.
9. Performing Organization Name and Address Techtran Corporation P.O. Box 729 Glen Burnie, Maryland 21061		11. Contract or Grant No. NASw-2485 13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address NATIONAL AERONAUTICS AND SPACE ADMINISTRATION Washington, D. C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of: "Vliyaniye Dipina na Induktsiyu Fenobarbitalom Tsitokhroma P_{450} i Stimulyatsiyu Mitoticheskoy Aktivnosti v Kletkakh Pecheni Krysy," Farmakologiya i Toksikologiya, Vol. 36, No. 5, 1973, pp. 597-599.

16. Abstract

The object of the research was to enquire into the influence exerted by dipine on the induction with phenobarbital of cytochrome P_{450} and on the stimulation of the mitotic activity in the rat's liver. Both of them are shown to be inhibited even in the case when the phenobarbital induction is instituted 60 days after a single injection of a non-cytostatic dose of the mutagen. The authors believe that changes occurring in the genetic system of the cell produced by dipine lie at the root of the cited phenomena.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified-Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	16	3.00

NASA-HQ

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V. V. Klimenko, L. Ye. Nemirovskiy and G. R. Mutovin[1]

This work is a study of the effect of dipine (tetraethylenimidepirazinedi- /597*
phosphoric acid) on phenobarbital induction of cytochrome P_{450}, which is the key enzyme of NADP·N dependent detoxificating system of the endoplasmatic reticulum of the hepatocytes. The work also contains a study of the effect of dipine on processes of stimulation of cellular division in the livers of rats.

The Methods of the Investigation. The experiments were carried out on 170 /598
mongrel male rats weighing from 150 to 170 g. Dipine was in a dose of 15 mg/kg, phenobarbital (the soluble sodium salt) in a dose of 60 mg/kg was administered intraabdominally in distilled water. On part of the animals, the liver was resectioned after the Higgens-Anderson method and the mitotic activity of the hepatocytes was determined. The rats were killed by decapitation. The liver was perfused with 0.25 M solution of saccharose through the lower inferior vena cava, and was then extirpated, weighed, and homogenized in a 0.25 M solution saccharose in ratio of 1:3. The homogenate was centrifuged at 9,000 g for a period of 15 minutes, at a temperature of 4°, to precipitate the mitochondria and nuclei. The content of cytochrome in the postmitochondrial precipitated fluid was determined on the SF-10 spectrophotometer (A. I. Archakov et al., 1969). The difference in optical density between the maximum of absorption at 450 nm and the minimum at 490 nm at a calculation of 1 mkg DNA phosphorus for 1 g tissue was the index of content of cytochrome P_{450}, which was expressed in arbitrary units:

$$\frac{\Delta D450 - 490}{\text{mmg}}$$

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*Numbers in the margin indicate pagination in the foreign text.

For estimating the mitotic activity, bits of liver were fixed in a mixture of ethanol-ice cold acetic acid (3:1). The mitotic activity of the hepatocytes was calculated using acetoorcein preparation (10,000 cells per animal).

The system of setting up the experiment consisted in the following. In the first series of experiments, the animals received dipine once, and then phenobarbital 4 times (with a days interval). After 24 hours, the material was fixed. In this case, the content of cytochrome and the mitotic activity in the liver were investigated. In the second and third series, 36 hours following 4 time administration of phenobarbital, resection of two-thirds of the liver was additionally carried out. Fixation was made 28 hours later and the mitotic activity of the hepatocytes was investigated. The time from the moment of administering dipine to the initiation of phenobarbital administration in series 1 and 2 was 3 days, in series 3, 60 days. The obtained results were processed statistically.

Results and Their Discussion. It was established that when phenobarbital was administered over the course of 4 days, the content of cytochrome P_{450} increased nearly 5 times in comparison with the level of the enzyme in the intact liver (see the drawing). The level of cytochrome 3 days after exposure to dipine alone only significantly exceeded the control figures.

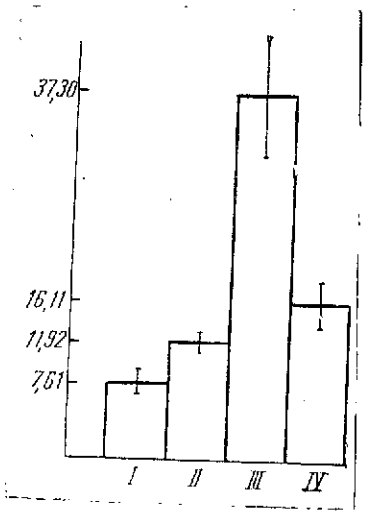
According to the data of A. S. Singin et al., 1970, dipine is completely eliminated from the rat organism on the third day. Therefore, we administered phenobarbital 3 days after dipine with the goal of levelling off the inducing effect of the latter on the detoxification system. In these experiments we observed a decrease in the level of cytochrome in comparison with the concentration of the enzyme in animals which had been administered only phenobarbital. The obtained facts indicate that the hepatocytes which are treated with dipine lose their capacity to respond to the stimulus of the inducer.

With 4 time administration of phenobarbital to the animals, the mitotic index in the liver significantly increased in comparison with the control (see the table).

It should be emphasized that the employed dose of dipine did not suppress mitotic activity in the intact liver. With the action of phenobarbital on rats

which had preliminarily received dipine, the number of mitoses approximated the original level.

With 4 time administration of phenobarbital, in combination with liver resection, maximum stimulation of cell division was achieved. However, if the animals were preliminarily administered a noncytostatic dose of dipine (15 mg/kg), a severe inhibition of the proliferative reaction was noted. A similar inhibition of the cellular cycle was obtained as well in the case in which /599 stimulation of mitotic activity of hepatocytes was carried out 60 days after the administration of dipine.



The Effect of Dipine (15 mg/kg) on Phenobarbital Induction of Cytochrome P_{450} In the Liver of the Rat. Verticals: content of cytochrome P_{450} (in arbitrary units). I, control; II, dipine; III, phenobarbital; IV, dipine + phenobarbital.

The fact that inhibition of regenerative proliferation is observed even 60 days following mutagenic treatment provides a basis to hypothesize that at the basis of the obtained phenomena lie changes in the genetic apparatus of the cell caused by dipine.

Conclusions

1. Dipine lowers the content of cytochrome P_{450} induced by phenobarbital in the microsomes of the hepatocytes.

2. The administration of dipine inhibits stimulation of mitotic activity in the liver caused by phenobarbital.

3. The biological effect of changes caused by dipine is maintained over the course of 60 days.

THE EFFECT OF DIPINE ON STIMULATION OF MITOTIC ACTIVITY IN THE RAT LIVER (M ± m)

Conditions of the experiment	Mitotic index (in %)		
	Intact Animals	Hepatectomized animals	
	1st series	2nd series	3rd series
Control	0,04±0,002	2,48±0,43	2,34±0,20
Dipine (15 mg/kg)	0,07±0,002	3,19±0,08	2,00±0,07
Phenobarbital (60 mg/kg)	0,27±0,04	5,56±0,10	3,95±0,15
Dipine + phenobarbital	0,08±0,003	0,58±0,03	0,67±0,03

Commas indicate decimal points.

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Translated for the National Aeronautics and Space Administration under Contract No. NASw-2485 by Techtran Corporation, P.O. Box 729, Glen Burnie, Maryland, 21061; translator, Samuel D. Blalock, Jr.